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Breeding Sugar Beet Root Maggot Resistance

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Breeding Sugar Beet Root Maggot Resistance

by

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"Sugar is nothing more nor less than concentrated sunshine" - George Rolph

1. Introduction

Breeding sugar beet (*Beta vulgaris* L.) for sugar beet root maggot (*Tetanops myopaeformis* von Roder; Diptera Order) resistance is an important topic for the sugar beet industry since most of the chemical control measures for this insect have used the same chemistry over the years; e.g. organophosphates, carbamates, pyrethroids and neonicotinoids. It is well known that the overuse of the same compounds can lead to pesticide resistance over time. Organophosphate resistance among other insect species of the Diptera order has already been documented (Campbell *et al.*, 2000b). Environmental safety concerns of pesticide usages are worrisome. The European Union ban of neonicotinoids adds uncertainty to the U.S. beet industry, with the fear that a similar action may be taken here in the U.S. (EPA, 2019).

During the 2016-2017 season, sugar beet production was valued at $1.64 billion (McConnell, 2020a). A 42% of yield loss has been observed in absence of control measures against the sugar beet root maggot attack (Campbell, 1998).

Sugar beet root maggot is an endemic pest of beets in the upper Midwest and western states of the U.S. and adjacent Canadian provinces. The largest U.S. sugar beet growing area is in the Red River Valley, which runs along the borders of Minnesota and North Dakota. Northwest growing area including Idaho, Washington State, Oregon and California is the second largest sugar beet producing area in the U.S. The third largest growing area includes Michigan followed by Upper Great Plains (Wyoming, Montana and western North Dakota) and Central Great Plains area (Southeast Wyoming, Colorado and Nebraska) (Figure 1; McConnel, 2020a).

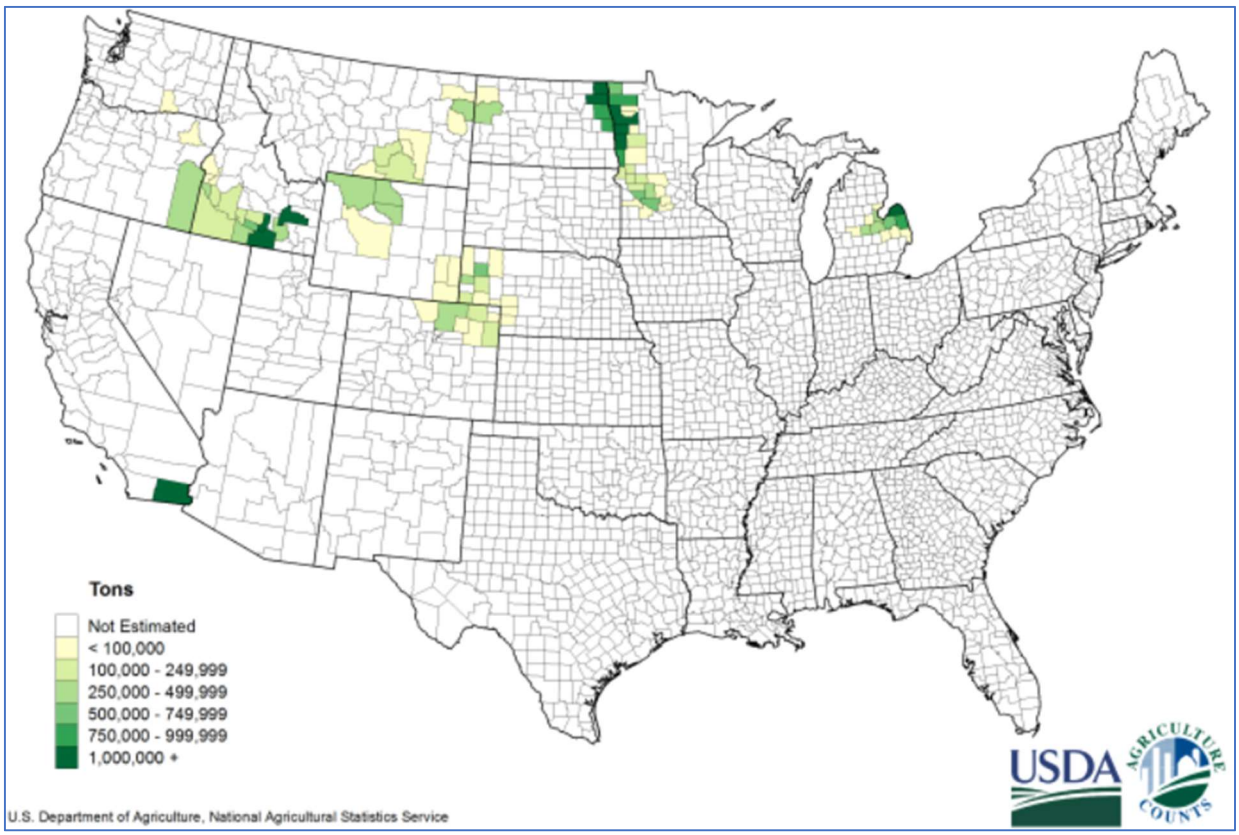

Figure 1 Sugar beet Production per Harvested Acre by County in 2018. U.S. Department of Agriculture. (USDA-NASS, 2018) https://www.nass.usda.gov/Charts_and_Maps/Crops_County/su-pr.php

Sugar beet root maggots feeding on tap roots lead to stand loss and wide-spread scar damages to the beets. The scar damages also open up the beet for opportunistic fungi to invade beets causing reduced beet sizes, sugar contents and recoverable sugar during pile storage and in severe cases death to the beet plants (Campbell *et al.*, 2006).

In 2009, the USDA-ARS and North Dakota State University released F1024, a sugar beet germplasm line resistant to sugar beet root maggot (Campbell *et al*., 2011). Before that in 1996 and 1998, two sugar beet root maggot resistant germplasm lines, F1015 and F1016, respectively (Campbell *et al*., 2000a), were released. Conventional breeding methods including mass selection have been used to identify sugar beet root maggot resistant germplasm. In St. Thomas,

ND, selections were made based on phenotypes of the feeding damage on the sugar beet root in naturally infested sugar beet root maggot nursery.

Very little is known about sugar beet root maggot biology and its interaction with the host plant. Puthoff and Smigocki (2007) studied the expression and regulation of sugar beet root genes in response to sugar beet root maggot feeding. The authors differentiated over 150 sugar beet root maggot responsive genes by comparing their expression levels between susceptible and moderately maggot resistant sugar beet lines in order to identify a marker associated with maggot resistance. Sugar beet root maggot resistance sources are limited and finding new genes for sugar beet root maggot resistance is very challenging. The intent of this review article is to understand the bottleneck of sugar beet root maggot resistance breeding and gain insight for conducting future research.

2. Sugar Beet Background

2.1 Sugar Beet Biology and Growth Habits

Sugar beet is herbaceous dicotyledon and a member of the Chenopodiaceae family. Sugar beets have two growth habits: annual and biennial. Annual habit is very common in wild beets; while, biennial growth habit is common to all commercial genotypes. In biennial commercial see production cultivars, the vegetative growth occurs in the first year followed by vernalization and the reproductive phase in the second year (Owen *et al.*, 1940). For breeding purposes, the sugar beet can be grown in greenhouse or winter nursery from seed for 2-3 months followed by placing the plants in a vernalization chambers at 5^{o}C and 8 h light for 12 weeks to induce bolting. After vernalization and photoinduction, the sugar beet stem starts to elongate, and inflorescences are developed. Beet plants display indeterminant growth. There are two types of breeding

germplasm pools determined by seed type: monogerm and multigerm. In the monogerm type, one single flower is developed in the bract axil; whereas in the multigerm type, two to seven flowers are developed in the bract axil (Artschwager, 1927). For controlled hybridization, all flower buds must remain closed before emasculation with the aid of a pair of tweezers. The emasculated flowers are protected from cross pollination by covering the stigmas with crossing bags, which are later replaced with the donor pollen bags.

The sugar beet is composed of compressed stem (crown), hypocotyl (neck) and true root (root). The sugar beet true root tissues are composed of parenchyma cells. Sucrose is stored in vacuoles of the parenchyma cells. The sugar beets terminate in a slender taproot, which could sometime be branched (Artschwager, 1926).

2.2 The U.S. Sugar Beet Production

In the United States, the first sugar factory was built in 1838 in Massachusetts; but the industry was not successful until 1870, when the sugar factories were established in California. Sugar beet crop was successfully introduced into the northern Midwest and Western States of the U.S. during 1950s (Francis, 2006). Currently, the major sugar beet producing states include North Dakota, Minnesota and Michigan that have long and cold winters. During the fall harvest, the sugar beet roots are piled outside. The low temperature slows the sucrose deterioration process. The other growing regions include the Northwest, Upper and Central Great Plains and Imperial Valley of California. Each growing area has its own sugar beet factory (Figure 2). Each market has its own payment formula and it is based on sugar beet tonnage and percentage of sugar content (Table 1). For the fiscal year 2017-2018, sugar beet yielded an average of 31.7 tons of roots per acre (McConnell, 2020a).

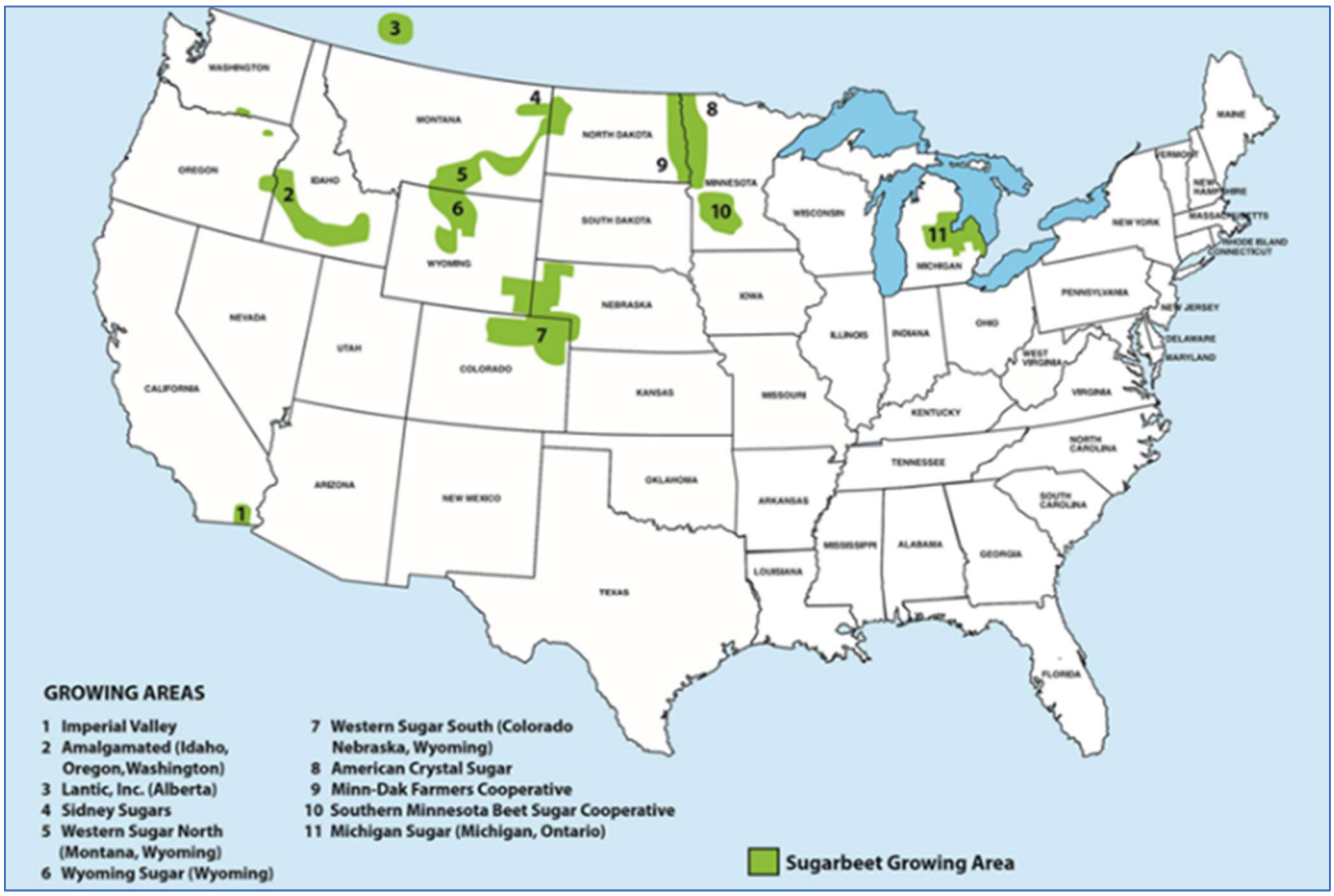

Figure 2 North America Sugar Beet Industry. Source: KWS, LLC.

In Minnesota, North Dakota, Idaho, Colorado, Montana, Nebraska and Wyoming, the sugar beets are most affected by sugar beet root maggot (Jaronski, 2020). The affected areas represent 84% of the all sugar beet planted area in the U.S. Minnesota and North Dakota alone count for 56% of the total sugar beet acreage (McConnell, 2020b).

Table 1. Sugar beet price in US$ per ton root by State in the U.S.

	Northwest				Midwest			Great Plains				National
Crop Year	California	Oregon	Washington	Idaho	Michigan	Minnesota	North Dakota	Montana	Nebraska	Colorado	Wyoming	Average
2007/08	43.60	36.90	36.90	36.90	36.00	45.20	46.30	39.10	40.40	36.00	40.20	42.00
2008/09	49.10	42.00	42.00	42.00	44.00	49.90	51.00	50.80	50.80	47.80	52.60	48.00
2009/10	63.40	45.10	---	45.10	60.80	49.80	51.90	53.40	54.60	53.30	53.90	51.50
2010/11	70.40	57.30	---	57.30	71.30	67.60	69.90	64.00	72.60	68.90	70.30	66.90
2011/12	66.30	65.40	---	65.40	87.70	68.30	60.80	71.90	72.50	68.40	74.60	69.40
2012/13	52.10	52.10	---	52.10	72.40	74.20	69.10	63.80	61.60	55.40	61.00	66.40
2013/14	43.00	40.00	---	40.00	53.60	52.60	44.90	39.40	37.80	35.60	37.20	46.46
2014/15	45.60	45.00	---	45.00	49.10	45.10	44.20	49.70	48.70	46.70	49.20	46.00
2015/16	46.90	45.50	---	45.50	45.30	46.40	48.30	52.90	51.30	51.00	54.70	47.20
2016/17	47.70	43.20	43.20	43.20	30.70	33.80	36.60	36.60	25.90	26.50	27.20	35.70
2017/18	47.70	40.20	40.20	40.20	38.30	42.50	44.20	41.30	33.70	31.60	37.30	41.20
2018/19												35.50

Source: USDA, NASS (2019). Agricultural Prices.

From 2015 to 2018, insecticide studies to control sugar beet root maggot were conducted in St. Thomas, ND (Boetel, 2020). The study compared granular insecticide and insecticide seed treatment combined with or without foliar insecticide applications at different concentrations. In this study, the application of granular and foliar insecticide significantly increased yield and revenue. The most effective combination of treatment applications was with organophosphate. Unfortunately, organophosphate is highly toxic to wildlife and humans through its contact or groundwater contamination. This highlights the importance of sugar beet root maggot resistance breeding for controlling this serious pest.

2.3 Sugar Beet Breeding Methods

Development of inbred lines for hybrid seed production is a challenge because of the presence of self-incompatibility in beet. However, identification of self-fertile genes (Owen, 1942; Savitsky H., 1952) facilitated the inbreeding process for generating homozygous monogerm and multigerm lines.

Mass selection is a commonly used method of breeding sugar beet. The breeders select lines with desirable traits and intercross them by open pollination. Seeds are bulked, and the selection cycle is repeated until the allele frequencies for desirable traits are enriched. Mass selection depends on heritability of traits because selection is based on phenotype only. Mass selection has contributed to the breeding for bolting resistance, crown height and low tare for reduced soil attachment to roots during harvesting, and for curly top, *Rhizoctonia* and Rhizomania resistance. However, mass selection is not efficient for quantitative traits such as sugar content and yield (Biaggi and Skaracis, 2005).

In recurrent selection, breeders select superior F_1 plants and cross among the F_1s in all possible combinations. Seeds are then bulked in equal proportions from the individual F_1s for the next selection cycle. The frequency of the superior allelic combinations is increased through selfing plants that show desirable trait phenotypes. The discovery of genes governing nuclear male-sterility in sugar beet by Owen (1952) facilitated the intermating of different genetic pools for recurrent selection and this method was termed as the male sterile facilitated recurrent selection (MSFRS).

Line breeding is used to evaluate and select superior lines based on progeny testing. The method includes selection of half- and full-sib families. Half-sib and full-sib family selection may be impractical since some sugar beet material produce low quantities of self-pollinated seed. However, once a desirable line is identified this obstacle can be overcome through vegetative propagation since sugar beet can be asexually propagated.

Interspecific hybridization is an important method for introducing disease and pest resistance genes to cultivars from wild species. The successful introgression of genes into sugar beets from *Beta procumbems* and *Beta corolliflora* includes introgression of cyst nematode *(Heterodera*

schactii) and beet curly top virus (BCTV) resistance genes, respectively (Savitsky, 1969; Savistky, 1975).

The breeding of sugar beets has been facilitated through *in vitro* culture; e.g., doubled haploid breeding; genomics; e.g., accessing genes for disease and insect resistance from new plant introduction lines; molecular breeding; e.g., marker-assisted selection (MAS) of traits using markers linked to quantitative trait loci (QTL) and major genes; and genetic engineering; e.g., transformation of genes into recipient sugar beet lines (Skaracis, 2005).

2.4 Sugar Beet Breeding Milestones

Sugar beet plants were descended from *Beta vulgaris* L. ssp. *maritima* (L.) known as sea beets. Early societies selected sea beets for the tenderness and sweetness of their leaves. Later, medieval societies shifted the use of beets to animal feed known as fodder beets that were grown for the root yield rather than leaves. The use of the sugar beet came to existence through the German effort to curb the sugarcane-based sugar monopoly created by Britain. Andreas Sigismund Marggraf presented his findings on sugar extraction from beets at the 1747 Berlin Academy of Science meeting. Franz Karl Achard followed up Marggraf's work and started mass selection based on root type of the fodder beets (Coons, 1936). Achard and his collaborators built the first sugar beet factory in 1802 and developed the "White Silesian" cultivar which became the progenitor of most of all cultivated sugar beets (Biancardi *et al.*, 2005). Successful introgression of genes from sea beets to sugar beets includes introgression of *Cercorpora* leaf spot and rhizomania resistance genes (Biaggi and Skaracis, 2005). *Cercorpora* leaf spot and rhizomania diseases are caused by *Cercospora beticola* and Beet Necrotic Yellow Vein Virus (BNYVV), respectively.

Sugar beet is a new crop compared to cereals and various other long-established crops. It benefited greatly from nineteenth and twentieth century breeding and implementation of agronomic techniques. One of the sugar beet breeding milestones is the development of monogerm seeds in the1950's (Savitsky, 1950). Victor Savitsky selected field plants with predominant monogerm flowers and through inbreeding created the monogerm SLC01 line, which was made available to sugar beet breeders worldwide. The development of monogerm seed eliminated hand-thinning to adjust plant density, a requirement for multigerm seeds. Some of the major sugar beet breeding accomplishments include: (i) introduction of wild beets as sources of diseases and pest resistance; e.g., *Hs1 pro-1* gene from *Beta procumbens* and *Beta webbiana* to confer resistance against the cyst nematode *Heterodera schachtii*, and monogenic *Holly* gene possibly originating from *B. maritima* sea beets to confer rhizomania resistance (Biancardi *et al.*, 2005); and (ii) mapping of the bolting tendency *B* locus containing the *B* gene that causes commercial varieties to bolt under extended low temperatures in the spring season (Walters *et al.*, 2013) and its associated markers (Abe *et al*, 1997); (iii) cytoplasmatic male sterility in monogerm lines and the development of maintainer O-type lines (Owen, 1945) allowing sugar beet breeding programs to take advantage of heterosis (Oldemeyer, 1957). Sugar beet has genetically been engineered to provide glyphosate resistance, commercially known as Roundup Ready Sugar Beets® through collaboration of Monsanto with KWS SAAT AG (USDA/APHIS, 2005).

3. Sugar Beet Resistance to Sugar Beet Root Maggot

Phenotyping for breeding physical resistance mechanisms against insects include selection of genotypes with hooked trichomes in beans that prevent aphid infestation (Gatehouse, 1991). Unfortunately, many other physical resistance mechanisms are governed by a large number of

genes or polygenes which are difficult to breed into a desirable cultivar (Gatehouse, 1991). Chemical resistance mechanisms include those that either interfere with insect development or cause mortality. In some sugar beet varieties, the resistance against sugar beet root aphid (*Pemphigus sp.*) can be a combination of the two-resistance mechanisms, physical and chemical barriers (Campbell, 1995).

In 1972 and 1973, potential sugar beet root maggot resistance was observed in field trials. Diverse lines from the *Beta* genus were evaluated (Callenbach *et al.*, 1972 and 1973). In the 1980s, field and greenhouse trials were conducted to evaluate inbred lines and their F_1 hybrids to determine levels of resistance, tolerance or susceptibility to the pest (Theurer *et al.*, 1982). In greenhouse tests, breeding materials were evaluated according to (i) damage ratings in sugar beet roots, (ii) the number of maggots, (iii) maggot weights and lengths, and (iv) fresh root and leaf weight of sugar beets. Based on five cycles of mass selection, levels of root maggot tolerance and susceptibility among the sugar beet populations were recorded (Theurer *et al.*, 1982).

Although sugar beet root maggot resistance was observed in the early 1970's, it took more than two decades to release root maggot resistant sugar beet germplasm lines, F1015 and F1016 (Campbell *et al*., 2000a). The new germplasm was developed as a result of collaboration between USDA-ARS and the North Dakota Agricultural Experiment Station. The F1015 line was derived from mass selection for low root maggot damage in the F1010 (PI 535818) root maggot susceptible sugar beet line. F1016 was derived from crosses of F1010 (PI 535818) with lines originated from an extinct sugar beet root maggot resistant line through collaborative research program between USDA-ARS and The Amalgamated Sugar Company. Mass selection was conducted among the progenies of the hybrids and F1016 with low root maggot damage was identified (Campbell *et al*, 2000a).

In 2009, a new germplasm line F1024 was released with sugar beet root maggot resistance by USDA-ARS and North Dakota Agricultural Experiment Station. F1024 was developed from a cross between F1016 with another breeding line 19961009H2 developed by USDA-ARS. The population derived from the cross was subjected to cycles of mass selection for low root maggot damage followed by half-sib selection, which led to development of F1024. The new germplasm was also improved for *Rhizoctonia* root rot and *Cercorpora* leaf spot resistance conferred by genes from the 19961009H2 breeding line (Campbell *et al*, 2011). *Rhizoctonia* root rot and *Cercorpora* leaf spot diseases are caused by *Rhizoctonia solani* J.G. Kuhn and *Cercospora beticola*, respectively.

The next germplasm released nearly a decade after the release of F1024, was F1043. The new germplasm line F1043 showed the same level of sugar beet root maggot resistance as previous germplasms. However, F1043 is not related to F1016 and F1024 and should be ideal for enhancing genetic variability of future breeding lines carrying sugar beet root maggot resistance genes (Campbell, 2017). F1043 was derived from a cross between sugar beet root maggot susceptible PI 610317 and the root maggot resistant PI 179180 line that produces red globe shaped beets. PI 179180 was selected in field trials by Callenbach *et al.* (1972; 1973). Eight cycles of mass selection followed by full-sib selection and then an additional mass selection in one full-sib family generated F1043.

Hybrid studies for sugar beet root maggot resistance were published in 2008. In a 2-year study, Campbell and Niehaus (2008) evaluated four entries of experimental hybrids, each originated from a different CMS susceptible line hybridized to F1015, one locally adapted commercial hybrid line and F1015. The treatments of insecticide and no-insecticide applications were also included in that study. Comparison among the entries without taking combining ability

and heterosis into consideration seemed to be difficult. Low to non-significant feeding damage ratings were observed between treatments and among the genotypes. Another factor to consider in that study was the low damage ratings in the first-year trial with no yield losses from the pest damages. In the second year, the damage ratings were moderate and yield losses were detectable. The study showed that the adapted commercial hybrid with no resistance to root maggot benefited the most from insecticide application as compared to the experimental hybrids and F1015. No significant yield improvement was observed from pesticide application versus non-pesticide application among F1015 and the four hybrids suggesting that the breeding for root maggot resistance is effective. Similar findings were also reported by Campbell *et al.* (2019).

The difficulty of selecting desirable genotypes in naturally root maggot infested nurseries resulted in low number of maggot resistant germplasm releases. The challenges of a naturally infested nursery include unpredictable weather and different insect species causing damages, indistinguishable from the ones by root maggot. High precipitation during maggot fly, egg laying and/or larval development could affect the infestation pressure and germplasm lines with beet root maggot resistance remain undetected. Different insect species such as wireworms (*Hemicrepidius memmonius*) and spring tails cause similar damages that could lead to mis-phenotyping a root maggot resistant germplasm line as susceptible. For example, wireworm feeding had interfered with the ratings for the beet root maggot resistance in F1024 (Campbell *et al*, 2011).

In order to curb the challenges in a naturally infested nursery, greenhouse assays have been developed. Theurer *et al.* (1982) compared resistant, susceptible and check inbred lines using a damage scale of 0 to 5 (0 = no damage; 5 = severe damage) following inoculation with either 25 or 50 root maggot eggs. The resistant inbred lines differed significantly from the susceptible and

check inbred lines. There was no significant difference in the host responses from the two inoculum levels. Daley *et al.* (2018) compared F1010 and F1024 lines using a damage scale of 0 to 3 with 0 = no damage; 1 = feeding damage on secondary roots; 2 = one to three feeding scars on tap root; 3 = four or more feeding scars on the tap root. The plants were caged with a pair of female and male flies. No evidence of damages was observed on F1024. However, a low damage level of 1 was observed on F1010. The susceptible lines did not show severe damages in any of the experiments, perhaps it was difficult to mimic the rhizosphere environment of a natural infested nursery in greenhouse (Iverson *et al*, 1984). Another difficulty in a controlled environment assay is the need for collecting larvae from infested fields and storing them. Storage conditions may have a negative impact on the pupation and fly emergence frequencies, which cause large variability in the number of maggots for the assay (Smigocki *et al*., 2006).

In 2006, Smigocki *et al.* established an *in vitro* bioassay. Roots of F1010, F1016 and F1043 seedlings were washed and placed on agar plates and inoculated with newly hatched first and second instar larvae. Second instar larvae were observed to be conglomerating and feeding on the susceptible line and dispersing from the resistant lines. The *in vitro* bioassay was rapid and one can evaluate the damages 48 h after larvae inoculation. In contrast, greenhouse assays can take 8 to 14 weeks to evaluate damages after inoculation with eggs (Daley, *et al.,* 2018; Theurer, *et al.,* 1982). The *in vitro* technique includes the challenges of preparing inoculum; maggot collection and storage. No report has been published thus far explaining the completion of the life cycle of the pest in a laboratory environment.

Most sugar beet germplasms are derived from wild beets originated from the Mediterranean coastline; whereas, sugar beet root maggot is native to North America. Therefore, sugar beets mostly lack inherent sugar beet root maggot resistance mechanisms. Sugar beet root aphid is also

native to the U.S. Sugar beet root aphid resistance is available and mapped to Chromosome I (Leijman, 2011). F1015, F1024 and F1043 are susceptible to sugar beet root aphid (*Pemphigus sp*) suggesting that different disease resistance mechanisms are involved in conferring resistance against sugar beet root aphid and root maggot (Campbell *et al.*, 2000a; Campbell, 2017).

To understand the mechanism of sugar beet root maggot defenses, gene expression in beet roots following root maggot feeding was analyzed by Smigocki *et al*. (2007). F1010 and F1016 were used in that study. They applied reverse transcription – polymerase chain reaction (RT-PCR) to measure the gene expression and suppression subtractive hybridization (SSH) to identify differentially expressed cDNAs. This study resulted in more than 150 expressed sequence tags (ESTs). Most of the ESTs are related to differential defense and stress responses of F1010 and F1016. In the F1016-specific EST collection, a gene *BvST1* encoding a serine protease inhibitor was identified. Protease inhibitor is a natural chemical defense mechanism used by plants against herbivorous insects. If the inhibitor is effective, insect toxicity is observed (Gatehouse, 1991). In sugar beet root maggot, serine proteinase is the main digestive enzyme present in the larval gut (Wilhite *et al.*, 2000). Its inhibition reduces the amount of amino acids available for larval growth and development (De Leo *et al.*, 2002). Therefore, the induced expression of *BvST1* following sugar beet root maggot attack could be used as a marker in selecting the putative sugar beet root maggot resistant germplasm lines.

4. Sugar Beet Root Maggot Background

4.1 Biology and Life Cycle

The sugar beet root maggot is the only known phytophagous insect species from the family Otinidae of the order Diptera. It is the only root maggot, which completes its life cycle in one

year with serious economic consequences to the sugar beet industry (Whitfield *et al.*, 1984). Before the introduction of sugar beet to North America, native weeds including common lambsquater (*Chenopodium album* L.), red root pigweed (*Amaranthus retroflexus* L.) and palmer amaranth (*Amaranthus palmeri* S. Watts) were hosts to this small two-winged fly (Figure 3) (Msangosoko *et al.*, 2018).

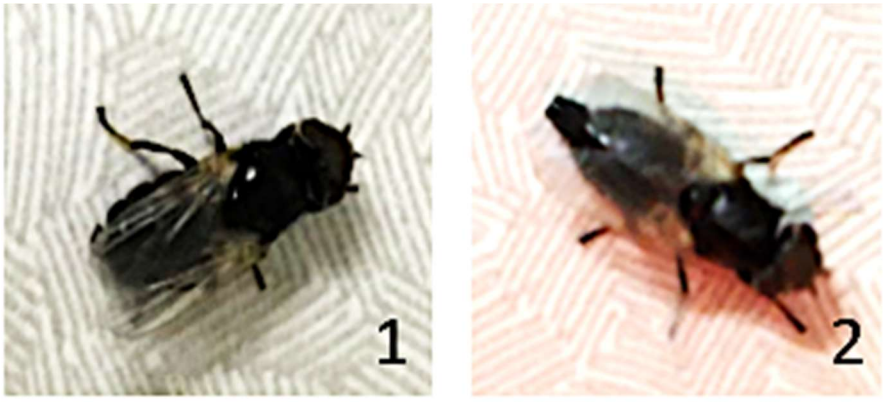

Figure 3 - Sugar beet root maggot fly. Male fly (1) and female fly (2) hatched on March 11, 2020. Betaseed, Inc, Shakopee Photo: Paloma Moe

The female fly lays egg clusters below the soil surface or in soil cracks next to the host plant. The egg clusters contain from 2 to 94 eggs (Figure 4; Harper, 1962). The larvae emerged from hatched eggs, scrap the surface of the sugar beet roots with their mouth hooks (Figure 5). The activity period of fly for mating lasts over 40 days (Lundquist, 1972). Depending on the fly infestation and the number of eggs laid, significant initial loss of stand can be observed.

However, the main yield loss comes from larvae feeding throughout the season (Campbell *et al.* 1998).

Figure 4 - Sugar Beet Root Maggot eggs. Betaseed, Inc, Shakopee. Photo: Paloma Moe

Figure 5 - Sugar beet taproot maggot feeding damage throughout the season. Field: St. Thomas nursery, St Thomas, ND. Photo: Carla Adamek, Betaseed, Inc., Shakopee.

In order to complete their life cycle (Figure 6), the host plant must supports the maggot's survival until third instar. The maggots cease feeding and tunnel themselves 2 to 14 inches into soil to diapause over the winter (Harper, 1962). During late spring and/or early summer, the overwintered maggot tunnels into soil surface to pupate and emerge as adults (flies) in late spring and/or early summer (Anderson, 1975).

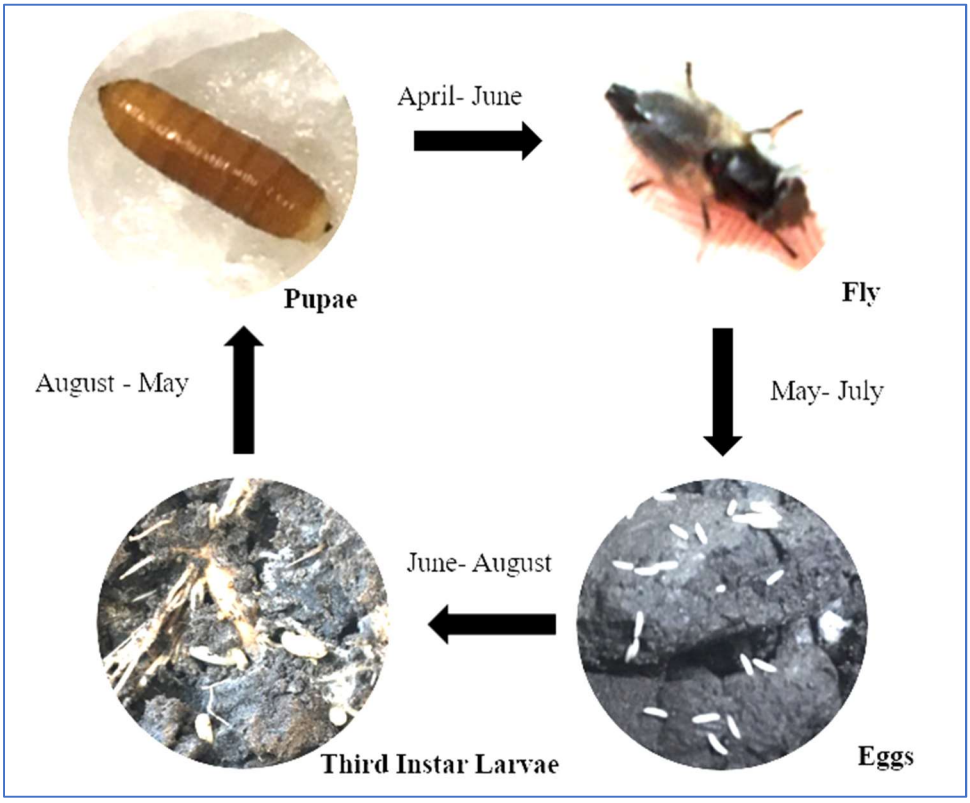

Figure 6 – Life Cycle of Sugar Beet Root Maggot. After egg laying and hatching, larvae feeds on the tap root and secondary roots from June to August, (Armstrong et al., 1998). Photo: Paloma Moe

The body length of the first, second and third instar larvae ranges from 0.75 to 2.1 mm, 2 to 3 mm and 3 to 11 mm, respectively; and the body-width of the three larval stages, first, second and third star, ranges from 0.2 to 0.5 mm, 0.4 to 0.8 mm and 0.8 to 2.0 mm, respectively. The body

color ranges from white to cream or light-yellow color as the larvae grow older (Figure 7; Bjerke *et al.*, 1992).

Figure 7- Sugar Beet Root Maggot third instar larvae after diapause. The third instar larvae were collected in July 30, 2018 St. Thomas, ND and stored at Betaseed, Inc, Shakopee. Photo: Paloma Moe

4.2 Field Damage Assessment

Yun (1972) discussed the importance of accurate and uniform root maggot assessment methods to be used across the industry. During 1970s, resistance breeding against sugar beet root maggot was not an option. The root maggot assessment was applied only to evaluate performance of insecticides. The chemical industry relied on stand loss, larval count and yield data to assess the damages. Yun (1972) developed a damage rating scale of 1 to 5, 0 = no damage to 5 = severe damages. Bickerstaff *et al.* (1977) reported a similar rating scale of 0 to 5, with 0 = no damage to 5 = severe damages. They reported a correlation coefficient of 0.95 between the number of maggots applied as inoculum and the damage ratings. Again, the rating scale was created to evaluate the insecticide performance.

Campbell (2005) modified the damage scale from 0 to 9 (Table 3) for their breeding program. Meanwhile, the ratings of Blickenstaff *et al.* (1977) were applied for determining the efficacy of insecticides among the commercial varieties.

Table 3. Sugar beet root maggot damage rating scale (Campbell, 2005)

Scale	Description 1	Description 2
0	No visual damage	
1	1 to 4 pinhead size scars	Minor damage
2	5 to 10 small cars	
3	Up to 3 large scars or scattered small scars	
4	Few large scars and/or numerous small scars	Moderate to heavy damage; detectable yield reduction.
5	Several large scars and/or heavy feeding on lateral roots	
6	Numerous scars with up to ¼ of root scarred	
7	¼ to ½ of root blackened by feeding scars	Severe damage; considerable yield reduction.
8	½ to ¾ of root blackened	
9	More than ¾ of root blackened	

5. Discussion and Conclusion

Breeding for sugar beet root maggot resistance has showed slow progress over the years. The application of pesticides is the most effective method of controlling this pest. However, safety concern of pesticide application, lack of new chemistry, and evolution of pesticide resistance have called for sugar beet root maggot resistance breeding.

Conventional breeding methods have not been able to deliver diverse sugar beet root maggot resistant cultivars or lines for production of commercial hybrids. Lack of sugar beet root maggot resistance in the sugar beet germplasm is the major bottleneck in breeding root maggot resistant sugar beet. Absence of desirable root maggot resistance is attributed to evolution of sugar beet and root maggot in two distinct geographical regions. The pest is endemic to the North America; whereas sugar beet was evolved from the wild sea beet in the Mediterranean region. Sugar beet seed companies currently offer seed treatments such as Poncho Beta® (clothinanidin and beta-cyfluthrin) for controlling this serious pest that causes yield suppression valued between $251 - $656/ha and a total annual yield suppression valued at $446 million in the U.S. (Boetel *et al*. 2010; McConnell, 2020a).

F1016 and F1024 are the most promising moderately root maggot resistant germplasm; and are most desired for mechanical harvesting because of their ideal root architecture. Complementary to current plant breeding effort, genetic engineering of sugar beets for insect and pest resistance could be feasible as has been demonstrated in maize and cotton. Smigocki *et al.* (2009) analyzed hairy roots transformed with *BvSTI* gene encoding a proteinase inhibitor protein against beet and fall armyworm and observed delayed development to high mortality of larvae. However, the costs of deregulation for an insect resistance transgene are very high and could also be controversial. The lawsuit against APHIS for deregulating Roundup Ready Sugar Beets® is a good example (USDA-Aphis, 2020). In addition, transgenic sugar beet root maggot resistance could have a niche market only as compared to broader economic importance of transgenic *Bacillus thuringiensis*-corn and -cotton crops that confer European corn borer (*Ostrinia nubilalis*) and bollworm (*Helicoverpa zea*) resistance.

In conclusion, more studies are required to better understand host and pest interaction allowing researchers to mimic natural rearing in a controlled environment for developing efficient screening protocols for identifying natural sugar beet root maggot resistance genes. With an efficient assay, it should be possible to compare regions of the genome of F1024/F1043 against F1010 to identify genomic regions containing root maggot resistance genes. Considering the lack of abundance in natural sugar beet root maggot resistance sources, one can opt to mutation breeding. Hohmann *et al.* (2008) developed a protocol to induce mutations in sugar beets using ethyl methanesulfonate (EMS) for targeting-induced local lesions in genomes (TILLING) to identify deficient bolting alleles in the *B* gene. Once an efficient screening system for identifying beet root maggot resistance is available, both targeted and random mutation breeding approaches can be pursued to identify novel maggot resistance genes.

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